The Ability of Guinea Pigs to Synthesize Carnitine at a Normal Rate From ε-*N*-Trimethyllysine or γ-Butyrobetaine In Vivo Is Not Compromised by Experimental Vitamin C Deficiency

Charles J. Rebouche

Experimental vitamin C deficiency in guinea pigs is associated with low carnitine concentrations in blood and some tissues. Ascorbic acid is a cofactor for two enzymes in the pathway of carnitine biosynthesis. The effect of experimental vitamin C deficiency on the ability of guinea pigs to synthesize carnitine was investigated in animals fed a vitamin C–deficient diet for 28 days. On days 19 to 28, supplements (0.5 mmol · kg body weight^{-1} · d^{-1}) of the carnitine precursors ε-*N*-trimethyllysine or γ-butyrobetaine were administered orally. Ascorbate-supplemented, ascorbate-deficient, and pair-fed (to ascorbate-deficient) animals showed an increase in the rate of carnitine biosynthesis (as estimated from measured rates of carnitine excretion) of 32 to 40 μmol · kg body weight^{-1} · d^{-1} following supplementation with ε-*N*-trimethyllysine. Likewise, animals in each experimental group showed an increase in the rate of carnitine biosynthesis of 41 to 50 μmol · kg body weight^{-1} · d^{-1} after supplementation with γ-butyrobetaine. These results indicate that scorbutic guinea pigs are able to synthesize carnitine at a normal or above-normal rate. For guinea pigs not given a carnitine precursor supplement, rates of free and total carnitine excretion for ascorbate-deficient (but not pair-fed) animals were threefold higher than for ascorbate-supplemented animals during days 19 to 28 of the feeding regimen. Thus, carnitine depletion in vitamin C deficiency likely is due to excessive urinary excretion of carnitine and not to a decreased rate of carnitine biosynthesis.

DEFICIENCY OF VITAMIN C in humans results in a variety of clinical manifestations, among which are fatigue and lethargy. Guinea pigs, which, like humans, require an exogenous source of vitamin C, develop secondary carnitine depletion in experimental vitamin C deficiency (see Rebouche[1] for a review). Because ascorbic acid is a required cofactor for two hydroxylation reactions in the pathway of carnitine biosynthesis, a number of investigators have proposed that secondary carnitine depletion results from a decreased rate of carnitine biosynthesis. Carnitine is required for efficient utilization of fat for energy production. Depletion of this nutrient may explain the symptoms of fatigue and lethargy that appear early in the development of scurvy. Experimental evidence derived from investigations in vivo[2-4] and with perfused liver from guinea pigs[5] seems to support the hypothesis that rates of carnitine synthesis are decreased in experimental scurvy, but the results are inconsistent both in reports from the same laboratory and from one laboratory to another.

Most recently, Alkonyi et al[4] provided evidence that the rate of carnitine excretion is greater in scorbutic guinea pigs and in guinea pigs pair-fed to scorbutic animals (and thereby food-restricted) as compared with normal guinea pigs. Thus, a more attractive hypothesis for the etiology of carnitine depletion in scorbutic guinea pigs (and food-restricted animals) is the excessive rate of renal loss of this amino acid. The objectives of this investigation are (1) to demonstrate that the normal rate of carnitine biosynthesis is not compromised in scorbutic and food-restricted guinea pigs, and (2) to show that the abnormally high rate of excretion of carnitine is the major contributor to carnitine depletion in these animals.

From the Department of Pediatrics, University of Iowa College of Medicine, Iowa City, IA.

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Address reprint requests to Charles J. Rebouche, PhD, University of Iowa, 100 Oakdale Research Park, Room A138, Iowa City, IA 52242-5000.

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MATERIALS AND METHODS

Materials

ε-*N*-Trimethyllysine and γ-butyrobetaine were synthesized as described previously.[6] All other chemicals were reagent-grade and obtained from commercial sources.

Animals and Animal Care

Male Hartley guinea pigs that weighed 232 to 352 g (at the time of arrival) were purchased from Central Iowa Cavies (Ames, IA) or SASCO (Omaha, NE). Animals were purchased and maintained in groups of six (two each for control, ascorbate-deficient, and pair-fed). Animals were housed individually in wire-bottom, stainless steel cages equipped with stationary stainless steel food cups, for 21 days (days −5 to 16). For the final 12 days (days 17 to 28) of the experimental regimen, animals were housed in metabolism cages (catalog no. 650-0350; Nalge, Rochester, NY). All guinea pigs were given free access to water and food (except animals in the pair-fed group) and were maintained on 12-hour light/dark cycles in a temperature-controlled room. All procedures that involved use of experimental animals were approved by the University of Iowa Animal Care and Use Committee.

Diets and Experimental Procedures

All guinea pigs were fed a commercially prepared, semipurified diet deficient in vitamin C supplied by Purina Mills (Richmond, IN; catalog no. 5711C-7). This diet is the same as the commercial Guinea Pig Chow 5025 diet supplied by Purina Mills, except that it was formulated by the manufacturer without added vitamin C. This diet contained 18% protein, 4% fat, and 16% fiber (manufacturer's analysis) and 30 to 40 mg vitamin C/kg diet, derived from natural ingredients (alfalfa, corn meal, soybean meal, etc.).

For the first 5 days (days −5 to 0), all guinea pigs were administered an oral vitamin C supplement (sodium ascorbate 5 mg and sucrose 2.5 mg per kilogram body weight per day, in a volume of water [in microliters], equal to half their body weight in grams). Supplements were administered through a plastic pipette

tip. During the experimental portion of the study, animals in control and pair-fed groups continued to receive the vitamin C and sucrose supplement daily, whereas animals in the ascorbate-deficient group were given only the sucrose solution.

Guinea pigs were acclimated to their living conditions and the diet during the first 5 days (days −5 to 0) of the study. During this time, body weights were recorded daily. On day 0, two animals were assigned to each of the three experimental groups: control, ascorbate-deficient, and pair-fed. Animals assigned to the ascorbate-deficient and pair-fed groups were matched as closely as possible by body weight. Animals in the pair-fed group were given the amount of food consumed on the previous day by their matched pair in the ascorbate-deficient group. Food intake and body weights were recorded daily from days 1 to 28. On days 17 to 28, complete 24-hour urine collections were obtained. Bacterial growth was inhibited during urine collection by addition of 1 mL 1-mol/L sulfuric acid to the collection vessel. Urine volumes were recorded, and aliquots were stored at −80°C for subsequent analysis.

On days 19 to 28, six to eight animals in each experimental group were administered daily, in addition to the sodium ascorbate and/or sucrose supplement, an oral pharmacologic dose of a carnitine precursor, either ϵ-*N*-trimethyllysine or γ-butyrobetaine (0.5 mmol · kg body weight^{-1} · d^{-1}). In addition, eight animals in each group received no carnitine precursor. Carnitine precursor supplements were administered in the same solution with sodium ascorbate and sucrose (control and pair-fed groups) or sucrose alone (ascorbate-deficient group).

On the morning of day 29, guinea pigs were anesthetized with ether, and blood was obtained by cardiac puncture. Animals were killed by asphyxiation, and the liver, kidneys, heart, and a portion of hindlimb skeletal muscle were removed, weighed, cut into small pieces, frozen in liquid nitrogen, and stored at −80°C for subsequent analysis.

Analytical Methods

Blood was collected into EDTA-treated plastic tubes, and plasma was obtained by centrifugation. Plasma was immediately divided into two aliquots. One aliquot was treated with 1 vol 10% wt/vol trichloroacetic acid, and the protein precipitate was removed by centrifugation. The supernatant was stored at −80°C for analysis of ascorbic acid. The second aliquot of plasma was stored untreated at −80°C for analysis of free and esterified carnitine, creatinine, total phosphorus, and uric acid.

Ascorbic acid concentrations in plasma and tissues were quantified by colorimetry after derivatization with 2,4-dinitrophenylhydrazine, as described by Omaye et al.[7] Nonesterified (free) and total (nonesterified and esterified) carnitine in plasma, urine, and tissues, and creatinine concentration in plasma and urine were quantified as described previously.[8] Glomerular filtration rate was estimated from creatinine clearance indexed to kidney weight.

Statistical Analyses

Comparisons were performed by ANOVA using the general linear models procedure.[9] First, data within groups (control, ascorbate-deficient, and pair-fed) that did not receive a carnitine-precursor supplement were compared. Second, groups of animals that received the carnitine-precursor supplements were compared with groups that did not receive the carnitine-precursor supplement, within each diet regimen (control, ascorbate-deficient, or pair-fed). Differences were identified using Tukey's studentized range test and corroborated by *t* tests. Differences were considered significant at P less than .05.

RESULTS

Growth responses to the ascorbate-deficient diet, ascorbate supplementation, and pair-feeding (Fig 1) were similar to results reported by other investigators.[10,11] Response to the ascorbate-deficient diet among individual guinea pigs was variable. Whereas the decrease in food consumption and weight loss was modest in some ascorbate-deficient animals, for others weight loss after day 16 was precipitous, and some of these animals did not survive through the end of day 28. Two animals in each of the three experimental groups that did not receive a carnitine-precursor supplement were killed on the morning of day 27 of the protocol, because the ascorbate-deficient animals were judged to be to ill to survive through day 28. One additional unsupplemented, ascorbate-deficient guinea pig was killed on the morning of day 25, likewise because it was judged to be too ill to survive another 24 hours. In the ascorbate-deficient group supplemented with ϵ-*N*-trimethyllysine, one guinea pig died on day 26 and one on day 28. No tissue or plasma metabolite data were obtained for these two animals. Early deaths of the most severely affected animals in the ascorbate-deficient group are reflected (as missing data) in the uneven patterns of weight loss from days 26 to 29 reported in Fig 1. Patterns of weight gain and loss for animals that received carnitine-precursor supplements (data not shown) were similar to those for animals not given a carnitine-precursor supplement.

Vitamin C concentrations in plasma and tissues were significantly lower in ascorbate-deficient animals than in pair-fed or control groups (Table 1). Differences between ascorbate-deficient and control animals were similar to those reported by Dunn et al.[5] In guinea pigs that did not receive a carnitine-precursor supplement, free and total carnitine concentrations were lower in plasma, liver, heart, and skeletal muscle but not in kidney of ascorbate-deficient

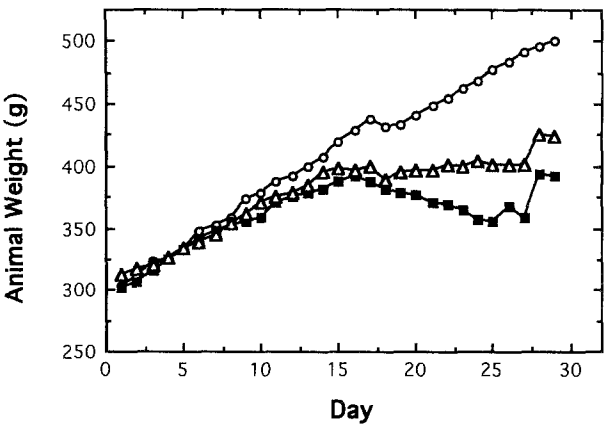

Fig 1. Weight gain or loss during 28-day feeding regimen. Animals in control (○) and pair-fed (△) groups were administered daily oral supplements of sodium ascorbate (5 mg · kg body weight^{-1} · d^{-1}). Guinea pigs in control and ascorbate-deficient (■) groups were given food ad libitum. Animal weights were recorded on the morning (beginning) of each day. Each point is the mean of weights for 8 animals, with the following exceptions: controls, n = 6 on days 28 and 29; ascorbate-deficient, n = 7 on days 26 and 27 and n = 5 on days 28 and 29; pair-fed, n = 6 on days 28 and 29.

Table 1. Ascorbate Concentrations in Plasma and Tissues

Supplement/Treatment	Plasma (μmol/L)	Liver (nmol/g)	Kidney (nmol/g)	Heart (nmol/g)	Skeletal Muscle (nmol/g)
None					
Controls (n = 8)	11.5 ± 4.28[a]	797 ± 157[a]	360 ± 84.3[a]	283 ± 35.5[a]	113 ± 23.9[a]
Ascorbate-deficient (n = 8)	3.10 ± 2.18[b]	76.9 ± 23.0[b]	52.4 ± 13.8[b]	40.5 ± 7.30[b]	32.3 ± 8.53[b]
Pair-fed (n = 8)	8.59 ± 3.83[a]	579 ± 167[c]	311 ± 74.9[a]	223 ± 72.0[a]	79.1 ± 9.04[c]
γ-Butyrobetaine					
Controls (n = 6)	15.8 ± 4.12	774 ± 80.4	418 ± 43.6	300 ± 53.8	121 ± 40.7
Ascorbate-deficient (n = 6)	3.31 ± 2.90	80.7 ± 36.7	65.6 ± 38.5	36.9 ± 22.8	30.2 ± 27.3
Pair-fed (n = 6)	8.95 ± 4.76	468 ± 169	348 ± 183	247 ± 94.5	116 ± 24.4*
ε-*N*-Trimethyllysine					
Controls (n = 8)	9.81 ± 3.60	648 ± 195	423 ± 84.4	232 ± 35.8	123 ± 47.7
Ascorbate-deficient (n = 6)	5.79 ± 3.88	141 ± 77.9	81.0 ± 28.6	42.8 ± 20.2	38.2 ± 28.6
Pair-fed (n = 8)	8.91 ± 4.62	608 ± 175	407 ± 49.7	208 ± 40.6	86.3 ± 34.7

NOTE. Values are the mean ± SD. Within the group of animals that did not receive a carnitine-precursor supplement, values with different superscript letters in the same column are significantly different ($P < .05$). For guinea pigs given carnitine-precursor supplements, concentrations were compared with those in similarly treated animals that received no carnitine-precursor supplement (*significant difference).

animals as compared with control animals (Table 2). Carnitine concentrations in plasma and tissues of pair-fed animals were generally intermediate between those of control and ascorbate-deficient animals. Supplementation of guinea pigs with carnitine precursors for 10 days did not consistently alter plasma or tissue free or total carnitine concentrations.

For guinea pigs that did not receive a carnitine supplement, rates of carnitine excretion by scorbutic animals were significantly greater on days 25, 26, and 28 than corresponding rates for pair-fed or control animals (Fig 2). The magnitude of differences in free and total carnitine excretion between ascorbate-deficient and control or pair-fed groups was similar on each of days 20 to 28. However, because of greater variance in data on days 20 to 24 and 27, differences were not significant on these days using the conservative Tukey's test for comparison. Average rates of total carnitine excretion for days 19 to 28 were as follows: control, 4.69 $\mu mol \cdot kg^{-1} \cdot d^{-1}$; ascorbate-deficient, 15.1; pair-fed, 5.74. Similarly, average rates of free carnitine excretion (days 19 to 28) were as follows: control, 3.01 $\mu mol \cdot kg^{-1} \cdot d^{-1}$; ascorbate-deficient, 10.1; pair-fed, 3.67. With respect to the comparison between control and ascorbate-deficient guinea pigs, these results are consistent with those reported by Alkonyi et al.[4] However, unlike Alkonyi et al, we did not observe a higher rate of carnitine excretion by pair-fed animals as compared with controls.

Following oral supplementation with the carnitine precursors ε-*N*-trimethyllysine or γ-butyrobetaine beginning on day 19, free and total carnitine excretion rates were significantly increased to approximately the same extent in control, ascorbate-deficient, and pair-fed guinea pigs (Fig 3). For guinea pigs that received the ε-*N*-trimethyllysine supplement, average rates of total carnitine excretion for days 19 to 28 were as follows: control, 39.5 $\mu mol \cdot kg^{-1} \cdot d^{-1}$; ascorbate-deficient, 55.4; pair-fed, 38.2. Thus, with ε-*N*-trimethyllysine supplementation, the rate of total carnitine excretion was increased, on average, during days 19 to 28 34.8 $\mu mol \cdot kd^{-1} \cdot d^{-1}$ for controls, 40.3 for ascorbate-deficient animals, and 32.5 for pair-fed animals. Increases in rates of free carnitine excretion were similar: 23.0 $\mu mol \cdot kg^{-1} \cdot d^{-1}$ for controls, 23.8 for ascorbate-deficient animals, and 20.5 for pair-fed animals.

Table 2. Carnitine Concentrations in Plasma and Tissues

Supplement/ Treatment	Plasma (μmol/L)		Liver (nmol/g)		Kidney (nmol/g)		Heart (nmol/g)		Skeletal Muscle (nmol/g)	
	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total
None										
Controls (n = 8)	43.4 ± 7.22[a]	56.2 ± 9.10[a]	1.22 ± 0.261[a]	1.55 ± 0.285[a]	1.47 ± 0.355[a]	1.83 ± 0.233[a]	4.09 ± 0.855[a]	7.16 ± 1.17[a]	1.36 ± 0.370[a]	2.77 ± 0.728[a]
Ascorbate-deficient (n = 8)	21.8 ± 8.31[b]	37.7 ± 16.2[b]	0.749 ± 0.245[b]	0.888 ± 0.265[b]	1.36 ± 0.206[a]	1.74 ± 0.237[a]	2.76 ± 0.707[b]	4.54 ± 1.19[b]	0.598 ± 0.439[b]	1.24 ± 0.890[b]
Pair-fed (n = 8)	33.5 ± 7.73[c]	51.5 ± 7.58[a]	0.758 ± 0.259[b]	0.911 ± 0.261[b]	1.39 ± 0.378[a]	1.70 ± 0.254[a]	3.63 ± 0.861[a]	6.69 ± 1.66[a]	0.957 ± 0.431[a]	1.93 ± 0.748[c]
γ-Butyrobetaine										
Controls (n = 6)	48.6 ± 2.50	63.8 ± 6.65	1.33 ± 0.406	1.67 ± 0.567	1.67 ± 0.318	2.16 ± 0.393	4.30 ± 2.35	8.40 ± 2.81	1.75 ± 0.508	3.13 ± 0.884
Ascorbate-deficient (n = 6)	26.4 ± 8.79	37.8 ± 10.9	0.858 ± 0.602	1.15 ± 0.782	1.24 ± 0.214	1.67 ± 0.266	3.37 ± 1.32	6.08 ± 1.77	0.931 ± 0.566	1.67 ± 0.977
Pair-fed (n = 6)	35.0 ± 13.7	54.2 ± 11.4	0.707 ± 0.496	0.881 ± 0.544	1.46 ± 0.546	1.89 ± 0.666	3.65 ± 1.13	6.59 ± 1.12	1.20 ± 0.768	2.37 ± 0.821
ε-*N*-Trimethyllysine										
Controls (n = 8)	33.3 ± 6.79*	48.6 ± 10.7	1.09 ± 0.369	1.32 ± 0.366	1.78 ± 0.475	2.54 ± 0.666*	3.83 ± 0.911	6.79 ± 1.79	1.08 ± 0.358	2.26 ± 0.370
Ascorbate-deficient (n = 6)	20.9 ± 6.52	37.8 ± 12.8	1.07 ± 0.605	1.23 ± 0.672	1.33 ± 0.321	1.94 ± 0.288	3.39 ± 1.02	5.69 ± 1.92	0.558 ± 0.242	1.44 ± 0.544
Pair-fed (n = 8)	27.0 ± 8.35	42.1 ± 13.6	0.829 ± 0.407	1.00 ± 0.457	1.64 ± 0.481	2.30 ± 0.591	3.62 ± 1.14	6.38 ± 2.00	0.882 ± 0.303	1.97 ± 0.687

NOTE. Values are the mean ± SD. Within the group of animals that did not receive a carnitine-precursor supplement, values with different superscript letters in the same column are significantly different ($P < .05$). For guinea pigs given carnitine-precursor supplements, concentrations were compared with those in similarly treated animals that received no carnitine-precursor supplement (*significant difference).

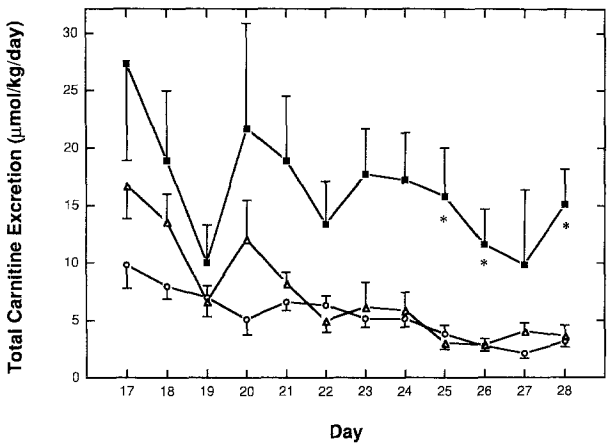

Fig 2. Rates of total carnitine excretion on days 17 to 28 of the feeding regimen (no carnitine-precursor supplement). On the morning of day 17, guinea pigs were placed in metabolic cages for complete collection of urine. (○), Controls; (■) ascorbate-deficient; (△), pair-fed. Each point is the mean of values for 8 animals, with the following exceptions: controls, n = 6 on days 27 and 28; ascorbate-deficient, n = 7 on days 25 and 26 and n = 5 on days 27 and 28; pair-fed, n = 6 on days 27 and 28. Vertical bars indicate standard error of the mean. *Significant difference ($P < .05$) from control group.

For guinea pigs that received the γ-butyrobetaine supplement, average rates of total carnitine excretion on days 19 to 28 were as follows: controls, 41.6 $\mu mol \cdot kg^{-1} \cdot d^{-1}$; ascorbate-deficient, 53.3; pair-fed, 41.2. Thus, with γ-butyrobetaine supplementation, average rates of total carnitine excretion were increased as compared with rates in unsupplemented animals by 36.9 $\mu mol \cdot kg^{-1} \cdot d^{-1}$ for controls, 38.2 for ascorbate-deficient guinea pigs, and 35.4 for pair-fed guinea pigs. Likewise, average rates of free carnitine excretion (days 19 to 28) also were increased, by 32.0 $\mu mol \cdot kg^{-1} \cdot d^{-1}$ for controls, 30.7 for ascorbate-deficient guinea pigs, and 29.1 for pair-fed guinea pigs provided the γ-butyrobetaine supplement as compared with unsupplemented animals.

Glomerular filtration rates, estimated from creatinine clearance indexed to kidney wet weight, were as follows (mean ± SD): controls, 0.307 ± 0.108 $mL \cdot min^{-1} \cdot g^{-1}$; ascorbate-deficient, 0.233 ± 0.100; pair-fed, 0.283 ± 0.115.

DISCUSSION

L-Carnitine ultimately is synthesized from L-lysine and L-methionine. After methylation of lysine residues in a variety of proteins and release of ε-*N*-trimethyllysine by protein hydrolysis, L-carnitine is synthesized from the latter amino acid by a sequence of four enzymatic reactions. Two of the enzymes that participate in this sequence, ε-*N*-trimethyllysine hydroxylase and γ-butyrobetaine hydroxylase, are α-ketoglutarate–requiring dioxygenases that also use Fe^{2+} and ascorbate as cofactors. Previous results from this laboratory demonstrated that humans and rats have a capacity to synthesize carnitine from γ-butyrobetaine at least 30- and 250-fold, respectively, greater than the normal rate of carnitine biosynthesis in these species, when sufficient precursor is provided orally.[12,13] Moreover, rats were shown to have an increase in the rate of carnitine biosynthesis of at least 100-fold when provided with oral supplements of ε-*N*-trimethyllysine.[14] Thus, these species apparently possess a large excess of enzymatic activity, and the ascorbate necessary to sustain that activity, relative to the capacity used for normal rates of carnitine biosynthesis. Although levels of ε-*N*-trimethyllysine hydroxylase activity have not been reported in guinea pig tissues, Englard and Carnicero[15] have reported that guinea pig liver contains approximately four times as much γ-butyrobetaine hydroxylase activity as rat liver. These data raised doubts about the validity of the conclusion that carnitine depletion in scorbutic guinea pigs is due to a reduced rate of carnitine synthesis from the precursors ε-*N*-trimethyllysine or γ-butyrobetaine.

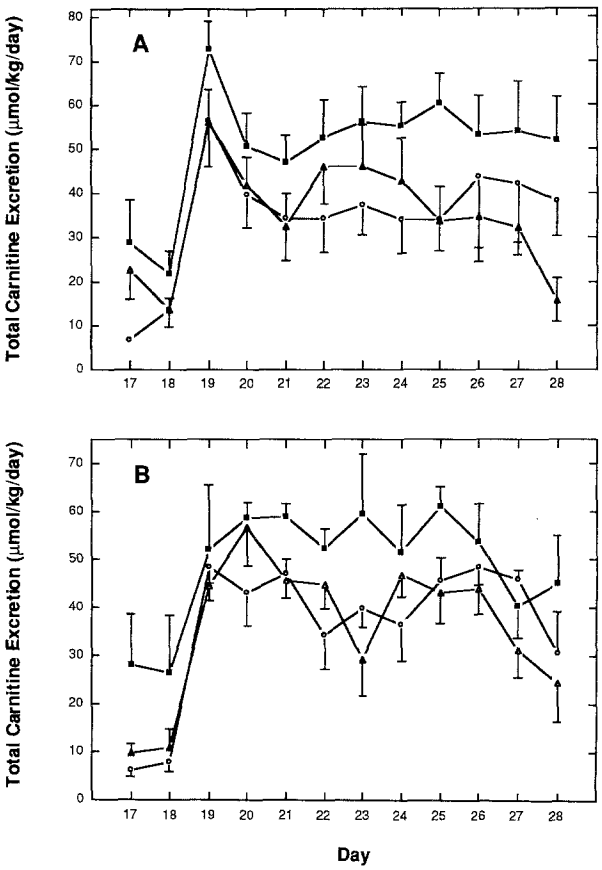

Fig 3. Rates of total carnitine excretion on days 17 to 28 of the feeding regimen. On the morning of day 17, guinea pigs were placed in metabolic cages for complete collection of urine. Supplementation with carnitine precursors was begun on the morning of day 19. (A) ε-*N*-Trimethyllysine supplement; (B) γ-butyrobetaine supplement. (○) Controls; (■) ascorbate-deficient; (△) pair-fed. (A) Each point is the mean of values for 8 animals, with the following exceptions: ascorbate-deficient, n = 7 on days 26 and 27 and n = 6 on day 28. (B) Each point is the mean of values for 6 animals. Vertical bars indicate standard error of the mean. For days 19 to 28, rates of total carnitine excretion with either ε-*N*-trimethyllysine or γ-butyrobetaine supplementation were significantly different ($P < .05$) from corresponding rates with no carnitine-precursor supplement given (Fig 2), with the following exceptions: control group on day 26, ε-*N*-trimethyllysine *v* no supplement and γ-butyrobetaine *v* no supplement; ascorbate-deficient group on days 27 and 28, γ-butyrobetaine *v* no supplement; and pair-fed group on day 28, ε-*N*-trimethyllysine *v* no supplement, and on day 23, γ-butyrobetaine *v* no supplement.

Close examination of results that support the above hypothesis, as well as inconsistencies in those results, also raises doubts about the validity of the conclusion. For example, in studies in vivo in scorbutic and normal guinea pigs in which [^{14}C]γ-butyrobetaine was infused into the vena cava, Nelson et al[2] first reported no difference in the conversion of the tracer into carnitine. In this study, the amount of γ-butyrobetaine infused was approximately 400 nmol. In 1 half-hour all of the tracer was converted to carnitine, which nominally yielded a rate of synthesis of 12.8 μmol · 100 g body weight^{-1} · d^{-1}, or approximately four times higher than the reported normal rate of carnitine biosynthesis (based on steady-state carnitine turnover) in rats.[16,17] On the other hand, in a later report from the same laboratory using the same experimental design, Thoma and Henderson[3] reported a rate of conversion of [^{14}C]γ-butyrobetaine to carnitine of 0.055 μmol per gram of liver in 30 minutes, after injection of 55 μmol substrate into the vena cava of scorbutic guinea pigs. Although this rate was 23% of the corresponding rate in normal guinea pigs, it nevertheless was equivalent to 264 μmol · 100 g body weight^{-1} · d^{-1}, or approximately 100 times the normal rate of carnitine biosynthesis in rats.

Dunn et al[5] perfused normal and scorbutic guinea pig livers with [1,2,3,4-^{14}C]γ-butyrobetaine and reported that this substrate administered in the perfusion medium was rapidly hydroxylated to carnitine in normal livers at a maximal rate of 0.15 μmol · g tissue^{-1} · h^{-1}. They stated, with regard to this finding, "That rate ... far exceeds the estimated rate of carnitine synthesis in the liver calculated from a reported daily turnover for carnitine in the rat of 2.3 [to] 2.6 μmol per 100 g of body weight...." Dunn et al[5] further reported that "carnitine production from [1,2,3,4-^{14}C]γ-butyrobetaine ... in livers of ascorbate-deficient animals was depressed by 51%...." Experiments reported by Nelson et al,[2] Thoma and Henderson,[3] and Dunn et al[5] thus show not a decrease in the normal rate of carnitine synthesis in scorbutic guinea pigs, but instead, a diminution of a grossly supranormal rate generated by provision of a large bolus of substrate.

In the present study, we have shown that scorbutic guinea pigs are able to increase the rate of carnitine excretion to the same extent as control animals when provided with oral supplements of the carnitine precursors ε-*N*-trimethyllysine or γ-butyrobetaine. At the same time, tissue carnitine concentrations were unchanged, which indicates a steady state in which rates of carnitine excretion reflect rates of carnitine synthesis. These results are interpreted to indicate that scorbutic guinea pigs have the capacity to maintain a normal or above-normal rate of carnitine biosynthesis. Thus, by inference, the residual ascorbate present in scorbutic guinea pigs must be sufficient to support the two dioxygenase reactions in the pathway.

However, results obtained in this study do not rule out the possibility that the rate of carnitine biosynthesis could be compromised by insufficient availability of the substrate ε-*N*-trimethyllysine. However, a mechanism for reduced availability of this amino acid is not readily apparent.

Results of this study are consistent with the hypothesis that excessive urinary excretion of carnitine contributes to carnitine depletion in vitamin C deficiency. During the last 10 days (days 19 to 28) of feeding the experimental diet (with and without ascorbate supplement), ascorbate-deficient guinea pigs excreted more than three times as much free and total carnitine as vitamin C–supplemented animals, despite lower plasma free and total carnitine concentrations in ascorbate-deficient animals. Excretion of carnitine by animals pair-fed to ascorbate-deficient guinea pigs but supplemented with vitamin C was not different from that of control animals, which indicates that decreased food consumption did not account for changes in the rate of carnitine excretion in vitamin C–deficient animals.

Consistent with previous reports (see Rebouche[1] for a review), we observed lower carnitine concentrations in some tissues (liver, heart, and skeletal muscle) of ascorbate-deficient animals as compared with controls. These differences persisted even after the rate of carnitine synthesis was increased by supplementation with carnitine percursors, which suggests that mechanisms underlying formation or maintenance of tissue to extracellular fluid carnitine concentration gradients may be compromised in experimental scurvy. Furthermore, higher rates of carnitine excretion by ascorbate-deficient guinea pigs as compared with controls or pair-fed animals, despite lower plasma carnitine concentrations, suggest either a decreased ability to reabsorb carnitine or an increased rate of glomerular filtration by ascorbate-deficient animals as compared with controls or pair-fed animals. The latter possibility was excluded by estimates of glomerular filtration rates that were not different among treatment groups.

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